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🌐 Latex beads behavior assay

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ABSTRACT

Protocol for testing latex beads aggregation after resuspension in different buffers. It allows determining how beads storage conditions can influence their ability to move freely through a membrane.

GUIDELINES

In the following assay a little amount of beads suspension will be eluted through a membrane. Don't pipette close one sample to each other, since it will lead to overlap of the developed elution fronts.

Beads separation by centrifugation can lead to difficult resuspension of them. Be sure that in each step beads are properly dispersed. Avoid excessive centrifugation time.

MATERIALS

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⊗ nitrocellulose membrane sheets size 210 mm × 297 mm thickness 200 μm Sigma Aldrich Catalog #Whatman® FF170HP Din A

⊗ Latex beads carboxylate-modified polystyrene fluorescent red 500nm average size. Contributed by users Catalog #L3280-1ML

- PBS-T Buffer pH = 7.4 (Tween-20 0.05%)
- Carbonate - Bicarbonate Buffer 100 mM pH = 9.6
- MES Buffer 50 mM pH = 5.9

- 2-20 μL micropipette
- 100-1000 μL micropipette
- 1000 μL micropipette tips
- 200 μL micropipette yellow tips
- Eppendorf tubes for microfuge (1.5-2 mL)
- Microfuge for eppendorf tubes
- Permanent Marker

OPEN ACCESS

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 We use this protocol and it's working

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BEFORE START INSTRUCTIONS

Prepare a piece of nitrocellulose paper big enough to hold the samples (4x4 cm will be enough).

Latex Beads Resuspension

- 1 Centrifugate 4 different aliquots of 40 μ L 2.5 %wt. latex bead stock suspension at 15.000 rpm for 3 minutes. After centrifugation remove the supernatant slowly.
- 2 Resuspend the beads in 100 μ L of the four different buffers: distilled water, PBS-T, Bicarbonate Buffer and MES buffer.
For resuspending beads repeated pipetting it's highly recommended, aspirating and blowing out in the eppendorf tube.
Label the different eppendorfs with the buffer added.

Latex Beads Migration

- 3 For beads migration test, wait 10 minutes and pipette 10 μ L of each bead suspension on a piece of nitrocellulose membrane. Separate each drop one to each other at least 8 mm.
- 4 Wait until the deposited sample has migrated completely, and circle on the plastic backing of the nitrocellulose membrane the outline of the sample eluted front.
Visible results of beads retention in the membrane should be visible at this point.

Latex beads reestablishment

- 5 Repeat step 1 and 2, resuspending all the beads in distilled water.
- 6 Repeat step 3-4 with the resuspended bead stocks.